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<p>(54) Title: NOVEL METHODS AND APPARATUS FOR IMPROVED FILTRATION OF SUBMICRON PARTICLES</p> <p>(57) Abstract</p> <p>The subject invention pertains to novel methods of filtration, novel methods for production of filters, and novel filters, for the efficient filtration of particles. The materials and methods of the subject invention are particularly advantageous for the filtration of submicron particles, for example, nanoparticles, and can utilize the electrostatic attraction between particles and the fibers of microporous filters, for example, polypropylene filters. The subject methods of filtration can lower the energy barrier between the particles and the filter surface and thus increase the deposition of particles on the surface of the filter. The methods and apparatus of the subject invention can be used to filter particles from many fluids including water and air. Advantageously, the subject surface modified filters can result in increased fluid flow, for the same pressure drop, compared to conventional filters.</p>		

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DESCRIPTIONNOVEL METHODS AND APPARATUS FOR  
IMPROVED FILTRATION OF SUBMICRON PARTICLES

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Background of the Invention

Waterborne gastroenteritis is an important public health problem in both developed and developing countries (Payment et al. 1991a, Payment et al. 1991b, Rao 1976). Many cases of human infections by waterborne enteric pathogens have occurred in the United States and throughout the world. In the late eighties, more than 50 waterborne disease outbreaks were reported (Levine et al.). Between 1991 and 1992, 17 states and territories in the United States reported 34 outbreaks associated with drinking water. These outbreaks caused an estimated 17,464 persons to become ill (Moore et al. 1993). In 1993, a waterborne outbreak of cryptosporidiosis occurred in Milwaukee. During this outbreak over 400,000 people had intestinal disease (MMWR, June 16, 1995). A recent report by the Academy of Microbiology (Colwell, R. R., 1996) indicated that our surface and drinking waters are no longer microbiologically safe. According to the report, there has been a rise in waterborne diseases worldwide. The annual social costs of waterborne mild gastrointestinal illnesses in the United States has been estimated to be over 22 billion dollars. Yearly reported disease cases in the United States attributed to contact with waterborne pathogens were estimated to be at a low of one million and a high of seven million (ENEWS staff, 1998). The deaths were estimated to be between 1000-1200 per year. The most common illness of water associated diseases is gastroenteritis; other illnesses include hepatitis, typhoid fever, mycobacteriosis, pneumonia, and dermatitis (Levine et al. 1991, Payment et al. 1994, Sherris et al, 1990). More efforts and improvements in drinking water treatment processes are needed to improve and maintain the quality and quantity of drinking water today.

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Deep bed filtration through particulate media is a commonly used method for removing particles from water (Bitton, 1994). This filtration process may not be efficient for the removal of microorganisms and relies either on the addition of flocculants to the influent water or the development of a biofilm on the surface of the filter media. Metallic hydroxides flocs (ferric hydroxide and aluminum hydroxide) have been used to treat water and waste water for many

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years (Bitton, 1994). These flocs adsorb microorganisms and remove them from the water following settling or filtration. However, combining flocculation with filtration has had limited success in producing flow-through filters for removing microorganisms from large volumes of water. There are potential drawbacks to this approach; the flocs may not be retained by the  
5 filters or clog the filters and greatly restrict the flow of water, thus requiring frequent backwashing (Bitton 1994).

Several studies have examined the possibility of modifying filtration particulate media to improve their ability to remove microorganism removal efficiency. The use of efficient filtering materials that remove microorganisms ameliorate the aesthetic and chemical quality  
10 of the water and reduce the need for subsequent disinfection. These modifications include coating of diatomaceous earth or micro porous filters with cationic polymers (Brown et al. 1974, Preston et al 1988), impregnation of coal with metallic hydroxides (Chaudhuri and Sattar, 1986), addition of positive charges to silica using organosilane derivative (Zerda et al., 1985), incorporation of metallic hydroxides into and on the surfaces of solids using *in situ* precipitation  
15 of metallic hydroxides (Farrah et al. 1985, Lukasik et al. 1996), adsorption of metallic flocs onto surfaces ( Edwards and Benjamin, 1989), and incorporation of metallic peroxides into microporous filters (Gerba et al. 1988) or diatomaceous earth (Farrah et al. 1991).

Numerous cases of disease transmission associated with bathing in recreational waters have been reported (D'Alessio et al. 1981, Hawley et al. 1973, Kee et al. 1994, Lenway et al.  
20 1989, MMWR 1997, and Turner et al. 1987). The responsible etiological agents could be either viral, bacterial or protozoan and the symptoms include diarrhea, nausea, vomiting and abdominal pain. It is likely that many cases of milder infections go unreported. Disinfection and filtration are common methods used to control pathogens in swimming pool water. Diatomaceous earth and sand filters are commonly used for swimming pools. Diatomaceous earth filters have the  
25 advantage of efficient removal of microorganisms (Brown et al. 1974), but are subject to frequent clogging, flow restriction, and their use can often raise health concerns. These health concerns are related to the possible role of diatomaceous earth particles in lung diseases. Sand filters have been extensively used in water filtration processes (Bitton, 1994). These filters remove relatively small numbers of pathogenic microorganism from water. Their performance  
30 can be improved by allowing the development of a biofilm on the sand. Also, efficient removal of microbes can be accomplished using coagulants (polymeric compounds or metallic hydroxides). Although useful for water and waste water treatment, these procedures may not be practical for swimming pools. Swimmers may object to the presence of coagulants and long ripening periods for filters may not be possible. Filtration of swimming pools is usually  
35 combined with disinfection. Chlorine is the most common disinfectant used in water treatment;

it is effective in killing a wide range of microorganisms. However, its use is limited to low concentrations since it is caustic and may produce toxic byproducts by reacting with organic materials.

The removal of particulate contaminants is very important for many applications including, for example, water reclamation, potable water treatment, water purification in microelectronics and pharmaceutical industries, and other point-of-use filters where ultrapure water is required. The efficacy of particulate contaminant filtration is dependent on several factors including particle size, physicochemical properties of the particles, and the collectors or filter media. For example, due to their large pore sizes, conventional filters cannot be used to filter submicron/nanoparticles or biological particles such as bacteria and viruses. Although large particles can be filtered by entrapment mechanisms, as the size of the particle decreases, particle removal becomes more difficult and thus other techniques may be necessary for efficient filtration. In particular, techniques which increase the interaction between the particles and the collectors can be used to enhance filtration efficiency.

Deposition of submicron particles onto collectors has captured the interest of many investigators over the years (Fitzpatrick *et al.* [1973]; Clint *et al.* [1973]; Spielman *et al.* [1974]; Rajagopalan *et al.* [1977a]; Rajagopalan *et al.* [1977b]; Onorato *et al.* [1980]; Oak *et al.* [1985]; Shields *et al.* [1986]; Russell *et al.* [1989]; Sisson *et al.* [1995]; Johnson *et al.* [1996]; Chang *et al.* [1990]). The main focus of these investigations has been on the interactions between the particles and the collectors.

Polypropylene is commonly used to make prefilters and filters because it is extremely inexpensive and very inert. A technique which could alter polypropylene filters such as to increase the interaction between particles and such filters would enhance the filtration efficiency of such filters.

#### Brief Summary of the Invention

The subject invention pertains to novel methods of filtration, novel methods for production of filters, and novel filters, for the efficient filtration of impurities. Such impurities include, but are not necessarily limited to, particles and chemical species. The materials and methods of the subject invention are particularly advantageous for the filtration of submicron particles, for example nanoparticles, and can utilize the electrostatic attraction between particles and the matrix of microporous filters. The subject methods of filtration can lower the energy barrier between the particles and the filter surface and thus increase the deposition of particles on the surface of the filter. The methods and materials of the subject invention can be used to filter particles and dissolved chemical contaminants from many fluids including water and air.

Advantageously, the subject surface modified filters can result in increased fluid flow, for the same pressure drop, compared to unmodified filters.

5 In a specific embodiment, because most natural materials are negatively charged (Rosen, M.J., 1988), a monomolecular layer of cationic surfactant is added to a microporous polypropylene filter in order to give the surface of the filter a positive charge. Advantageously, the adsorption of negatively charged and/or neutral particles on these surface modified filters and the resulting filtration efficiency are enhanced. These surface modified filters can be useful for removing bacteria, viruses, and nanoparticles in waste water treatment, and can also be useful in resource recovery processes.

10 In an alternative embodiment, a monomolecular layer of anionic surfactant can be added to a microporous polypropylene filter in order to give the surface of the filter a negative charge, resulting in enhanced filtration efficiency for positively charged and/or neutral particles. In an additional embodiment, a monomolecular layer of nonionic surfactant can be added to a microporous polypropylene filter, resulting in enhanced filtration efficiency for positive, negative, and/or neutral particles.

15 In a further embodiment, the subject surface modified filters can be stacked, resulting in a further increase in filtration efficiency. Specifically, surface modified filters having a positive charge, negative charge, or neutral charge can be stacked in a variety of combinations to more efficiently filter target particles, for example, negative, positive, and neutral particles simultaneously. Filters made of materials other than microporous polypropylene can also be modified by the addition of surfactants or polymers in accordance with the subject invention.

20 The subject invention also pertains to novel methods of coating filter media, novel filter media, for the efficient filtration of chemical and biological contaminants from fluids. The materials and methods of the subject invention are particularly advantageous for filtration of ions, particulates, bacteria, viruses, protozoan parasites, fungus, yeast, and other submicron particles from aqueous and gaseous systems. The subject coatings can be applied to a variety of filter media, for example woven and unwoven filters or fabrics, fiberglass, fiberglass air filters, polypropylene, cellulose, sand, diatomaceous earth, fine sand, gravel, and any particulate filter media.

25 In a specific embodiment, *in situ* precipitation of metallic hydroxides and/or metallic oxides can be utilized to coat filter media. The coated filter media can be useful for removing, for example, bacteria, viruses, protozoan parasites, fungus, organic and inorganic chemicals, and/or dust. The subject materials and methods can be utilized in, for example, survival or personal water purification devices, household water filters, air filters, water reclamation filter

media, water remediation processes, soil remediation processes, viral removal filters, and/or protective clothing.

#### Detailed Disclosure of the Invention

5           The subject invention pertains to novel methods of filtration, novel methods for production of filters, and novel filters, for the efficient filtration of impurities in a fluid. The materials and methods of the subject invention are particularly advantageous for the filtration of submicron particles, for example nanoparticles, and can utilize the electrostatic attraction between particles and the matrix of a filter. The subject methods of filtration can lower the  
10   energy barrier between the particles and the filter surface and thus increase the deposition of particles on the surface of the filter. The methods and apparatus of the subject invention can be used to filter impurities from many fluids including water and air. Advantageously, the subject surface modified filters can result in increased fluid flow, for the same pressure drop, as compared to unmodified filters.

15           In a specific embodiment, because most natural particles are negatively charged (Rosen, M.J., 1988), a thin layer of cationic surfactant is added to a microporous polypropylene filter in order to give the surface of the filter a positive charge. In a specific embodiment, this layer can be monomolecular. Advantageously, the adsorption of negatively charged and/or neutral particles on these surface modified filters and the resulting filtration efficiency are enhanced.  
20   These surface modified filters are useful for removing bacteria, viruses, and nanoparticles in waste water treatment, and are also useful in resource recovery processes.

          In an alternative embodiment, a thin layer of anionic surfactant can be added to a microporous polypropylene filter in order to give the surface of the filter a negative charge, resulting in enhanced filtration efficiency for positively charged and/or neutral particles. In an  
25   additional embodiment, a thin layer of nonionic surfactant can be added to a microporous polypropylene filter, resulting in enhanced filtration efficiency for neutral particles. These layers may be monomolecular.

          Thus, in alternative embodiments, cationic polymers can be used to coat filter surfaces, resulting in increased filtration efficiency of negative and/or neutral particles; anionic polymers  
30   can be used to coat filter surfaces, resulting in increased filtration efficiency of positive and/or neutral particles; and nonionic polymers can be used to coat filter surfaces, resulting in increased filtration efficiency of negative, positive, and/or neutral particles.

          The subject invention is applicable to many types of prefilters and filters, including polymeric filters. In a preferred embodiment, the subject filters are microporous polypropylene  
35   filters, which are inexpensive and very inert.

Advantageously, the methods and apparatuses of the subject invention allow the monitoring of the charge neutralization of the filter surface to determine when the filter needs to be replaced. This monitoring can be accomplished by, for example, measuring the zeta potential of a filter using, for example, a streaming potential apparatus.

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Surfactants and polymers which can be utilized in the subject invention include the following:

Cationic Surfactants

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C<sub>20</sub> TAB - C<sub>26</sub> TAB,  
wherein TAB is Trimethyl ammonium bromide

Anionic Surfactants

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Dicetyl phosphate  
Phosphatidic acid

Cationic Polymers

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Poly (4-vinyl pyridine)  
Chitosan (Poly(d-glucosamine))  
Polyethylenimine

Anionic Polymers

25

Poly (acrylic acid)  
Poly (butyl acrylate/acrylic acid)

Others

30

Hydrophobically modified cationic polymers  
Hydrophobically modified anionic polymers  
Zwitterionic surfactants (both - and + groups)  
such as lecithin (Soya Lecithim)

Nonionic Polymers

Polyvinyl alcohol

35 although, it is understood that other surfactants and polymers can also be utilized within the scope of the subject invention.

In a further embodiment, the subject surface modified filters can be stacked resulting in a further increase in filtration efficiency. Specifically, surface modified filters having a positive charge, negative charge, or neutral charge can be stacked in a variety of combinations to more efficiently filter target particles, for example, negative, positive, and neutral particles simultaneously. Filters made of materials other than microporous polypropylene can also be modified by the addition of surfactants or polymers in accordance with the subject invention.

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The subject invention also pertains to novel methods of coating filter media, and novel filter media, for the efficient filtration of chemical and biological contaminants from fluids. The

materials and methods of the subject invention are particularly advantageous for filtration of chemical species, particulates, bacteria, viruses, protozoan parasites, fungus, yeast, and other submicron particles from aqueous and gaseous systems. The subject coatings can be applied to a variety of filter media, for example woven and unwoven filters or fabrics, fiberglass, fiberglass air filters, polypropylene, cellulose, sand, diatomaceous earth, fine sand, gravel, activated carbon, activated charcoal, and any particulate filter media.

The subject invention also relates to the precipitation of metallic (hydr)oxides on a filter matrix, the resulting filters, and method of filtering a fluid utilizing such filter. In a specific embodiment, *in situ* precipitation of metallic hydroxides and/or metallic oxides can be utilized to coat filter media. The coated filter media can be useful for removing, for example, bacteria, viruses, protozoan parasites, fungus, organic and inorganic chemicals, and/or dust. The subject materials and methods can be utilized in, for example, survival or personal water purification devices, household water filters, air filters, water reclamation filter media, water remediation processes, soil remediation processes, viral removal filters, and/or protective clothing.

In order to precipitate metallic (hydr)oxides on a filter matrix, where metallic (hydr)oxides refer to metallic hydroxides and/or metallic oxides, the filter matrix can, for example, first be wet with a metallic chloride solution. Other solutions can also be used, such as a metallic sulfate solution. Once the wetted filter matrix dries a basic solution can be applied. Examples of such basic solutions include ammonium hydroxide, sodium hydroxide, and potassium hydroxide. Other strong base solutions which raise the pH can also be used. Preferably, the pH of such basic solution is at least pH 9, and more preferably at least pH 11. Once the filter matrix dries again, the filter media having a coating of metallic (hydr)oxides on the surface is ready for use.

In a preferred method for applying the metallic chloride and ammonium hydroxide solutions, the subject filter media particles are preferably heated to an elevated temperature. Applying the coatings while the filter media is at elevated temperatures is particularly advantageous for sand, diatomaceous earth, and particulate filter media. For example, in range of 60°C to 100°C, more preferably 70°C to 95°C, even more preferably 75°C to 85°C, and most preferably about 80° C. In addition, preferably the filter media can also be agitated mechanically, for example on a vibrating platform. The agitation helps to encourage uniformity of the coating which is to be applied. The heating process can involve the use of, for example, infra red radiation, the blowing of hot air on to the vibrating particles, microwaves, or any other heating mechanism which can be incorporated with the subject coating process without interference. Once the media is heated, and preferably while being agitated, a metallic chloride solution can be applied to the media's surface. In a specific embodiment, a mist of a preheated

solution at approximately the same temperature as the filter media (e.g., about 80°C) of 0.25M ferric chloride and 0.5 M aluminum chloride in a 50 % ethanol solution is sprayed onto the vibrating particles. Preferably, the solution is sprayed in short bursts to avoid excessive wetting of the surface and to allow even drying. Heating and agitation can be continued throughout the spraying process. The misting is preferably continued until the surface of the solid substrate is evenly treated and fully coated with the metal chloride solution. It should be noted that a variety of methods can be used to apply the metallic chloride solution.

The coating is then allowed to dry. In order to dry quickly and evenly, heating and agitation can be continued. Excessive heating should be avoided in order to avoid the corresponding metallic oxides. The coated material tends to turn a yellowish coloration when coated appropriately. The solids can then be coated, for example sprayed by a mist or exposed to saturated vapors of ammonium hydroxides, by a 3.0 M ammonium hydroxide solution. A 3.0 M ammonium hydroxide solution is preferred. Again, the coating is preferably applied while the media is being heated and agitated. This forms the corresponding precipitate of metallic (hydr)oxides on the surface. The coated solids can then be rinsed in deionized water and dried.

The filter media is then ready for use. The metallic chloride solution can have a range of molarities. Preferably the ferric chloride is in the range of 0.1M to 2.0 M, more preferably 0.2M to 0.4M, and most preferably about 0.25M, while the aluminum chloride is preferably in the range of 0.1 M to 2.0 M, more preferably 0.2 M to 0.8 M and most preferably about 0.5M. The subject metallic (hydr)oxide coating can be generated utilizing metallic sulfates, such as Aluminum sulfate and/or Ferric sulfates.

The metallic chloride solution can be in, for example, 100% water, however ethanol is preferably added to speed the drying process. It was found that 50% ethanol - 50% water worked well. As mentioned heating was stopped after the metallic chloride solution dried to avoid the coating going to the oxide. A dark brown or black color may indicate oxide formation. Other coatings can be applied, such as magnesium peroxide, silver chloride, and Manganese oxide. As may also be described in further examples, the metallic chloride and ammonium hydroxide coatings can be applied in a variety of ways, for example immersion of the filter media is appropriate solutions or contact of filter media with saturated vapors. It was found that misting provides a uniform coating, but other application techniques, for example immersion, may be more practical for large volumes.

In a specific embodiment, fiberglass filter media can be precipitated with metallic (hydr)oxides. This embodiment is particularly advantageous for porous filters where misting can adequately wet the filter matrix. Heated air can be passed through the filters in order to

raise the temperature of the substrates to be coated to about 80°C. A mist of a solution preheated ( about 80°C) of 0.25M ferric chloride and 0.5 M aluminum chloride in a 50 % ethanol solution can then be sprayed onto the filter surface. Other combinations of metallic chlorides and/or sulfates can also be used. The hot air can continually be blown through it while the solution is sprayed. The solution is preferably sprayed evenly and sporadically to allow even drying. Heating can be continued throughout the spraying process. The surface of the solid substrate can be evenly treated and fully coated with a dry film of, for example, the metal chloride or metal sulfate. Again, excessive heating should be avoided in order to avoid forming the corresponding metallic oxides. The coated material may sustain a yellowish brown coloration when adequately coated. A basic solution can then be applied. For example, the solids can then be sprayed by a mist of a 3.0 M ammonium hydroxide solution or exposed to saturated vapors of ammonium hydroxides while being heated. This forms the corresponding precipitate of metallic (hydr)oxides onto the surface. Drying by the passing of hot air can be continued until the filter is dry. The coated solids can then be rinsed in deionized water and dried. After drying, they are ready for use.

The subject coating methods can also be applied to would fiber filters such as those used under a sink for filtering tap water. These filters are often made from a dense filter media such as cellulose, where misting can be inadequate to wet the filter matrix. For this type of filter, the substrates can be heated to preferably about 80° C. A preheated (about 80°C) solution of 0.25M ferric chloride and 0.5 M aluminum chloride in a 50 % ethanol solution can be passed through the filter. The filter is preferably saturated with the solution. A contact time of 10 min allows adequate contact, although longer or shorter periods can also be utilized. Heating can be continued during the contact time. Hot air can then be passed through the filter to flush the excessive solution. The passing of the hot air can be continued until the filter is thoroughly dried. The filter should now be evenly treated and fully coated with a dry film of the metal chloride. Excessive heating should be avoided in order to avoid the corresponding metallic oxides. The coated material can sustain a yellowish-brown coloration when adequately coated. A solution of a 3.0 M ammonium hydroxide preheated to about 80°C can then be passed through the filter rapidly or saturated vapors of ammonium hydroxides can be passed through the filters while being heated. This forms the corresponding precipitate of metallic hydroxides on the surface. Hot air can then be passed through the filter to remove excessive liquid and dry the filter. The coated solids can be rinsed in deionized water and dried. Once dry the coated solids are ready for use.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

5    Example 1 – Filtration of Nanoparticles with Surfactant Treated Polypropylene Filters

The filters used in this example are melt-blown microporous polypropylene disk filters with a diameter of 25 mm (Millipore, MA). The average pore sizes are 0.6  $\mu\text{m}$  (AN06), 1.25  $\mu\text{m}$  (AN12), 2.5  $\mu\text{m}$  (AN25), 5.0  $\mu\text{m}$  (AN50), and 10.0  $\mu\text{m}$  (AN1H). These filters were treated with a two-tailed water insoluble cationic surfactant, dimethyldioctadecylammomium bromide (DDAB), by first dissolving the surfactant in methanol and then soaking the filters in the methanol solution (4 ml per filter) for 3 hours. After the 3-hour treatment, the filters were vacuum dried overnight. Before using them for filtration experiments, the filters were flushed with 30 ml of distilled water to remove excess and/or loose surfactants from the filter surface. The average pore diameters were supplied by the manufacturer. Dimethyldioctadecylammonium bromide was purchased from Sigma Chemical Company (St. Louis, MO) and used without further purification.

The nanoparticles were purchased from Bangs Labs, Inc. (Carmel, IN). The negatively charged particles, P(S/A/V-COOH), are composed of polystyrene, an acrylic polymer (unspecified), a vinyl group (unspecified polymerizable group), and a functional carboxylate group. The average diameter and surface charge density of these nanoparticles are  $60 \pm 3.6$  nm and 365  $\mu\text{eq/g}$ , respectively, and 197 nm (no standard deviation given) and 141  $\mu\text{eq/g}$ , respectively. The positively charged particles are quaternary ammonium modified polystyrene particles (P(S/Quat Ammonium)) with an average diameter of 200 nm and a surface charge density of 121  $\mu\text{eq/g}$ . Particle concentrations (wt% solids) were measured by UV absorbance with a Hewlett Packard 8453 UV/VIS spectrophotometer at a wavelength of 255 nm.

Qualitative comparison of the degree of DDAB coating on the filters was done through contact angle measurements. AN06 (0.6  $\mu\text{m}$ ) filters were treated with different concentrations of DDAB according to the procedure mentioned above. After the filters were rinsed with 30 ml of distilled water, they were cut into four strips. The strips were then submerged onto a rectangular piece of PMMA (4 mm in thickness) in a 50 ml beaker (Pyrex No. 1000) containing 20 ml of distilled water. A drop, approximately 1.5  $\mu\text{l}$ , of 1,1,2,2-tetrabromoethane was placed on top of the filter with a microsyringe. After the droplet was allowed to spread on the filter for 1.5 minutes, the contact angle of the droplet was measured with a contact angle goniometer. Six readings at different locations on the filter surface were taken with each of the filters to get an average contact angle value. Zeta potential of the filters were measured with the Brookhaven

BI-EKA Electrokinetic Analyzer on loan for equipment evaluation (Brookhaven Instruments Corporation, New York).

For particle adsorption experiments, the filters, both untreated and treated AN06, were rinsed with 30 ml of deionized distilled water. Each filter was then soaked in 25 ml of a particle suspension containing 0.012 wt% solids ( $2.8 \times 10^{10}$  particles/ml) of 197 nm P(S/A/V-COOH) or 200 nm P(S/Quat Ammonium) for 30 minutes. The filter was then removed from the suspension and rinsed in 100 ml of distilled water and then vacuum dried overnight before it was coated with gold for scanning electron microscopy. SEM pictures were taken with a Hitachi S4000 Field Emission Scanning Electron Microscope (Tokyo, Japan). Particle analysis was performed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/ni-image/>).

Filtration was accomplished by using a stainless steel filter holder, a 12-ml glass syringe and a syringe pump with variable speed control (Dual Infusion/Withdrawal Pump, Model 944, Harvard Apparatus Co., Millis, Mass). For each experiment, 10 ml of particle suspension was filtered through each filter at a constant flow rate of 9 ml/minute. The filtrate was collected in a 11-ml glass vial and then transfer to UV/VIS cell for concentration measurement.

Microporous polypropylene filters were coated with a monomolecular layer of dimethyldioctadecylammonium bromide (DDAB) to give them a positively charged surface. DDAB was chosen because of the two hydrocarbon chains that can hydrophobically bond to the surface of the polypropylene and make this surfactant insoluble in water. Contact angle was used as a measure of the amount of DDAB adsorption at the surface of the filters. For smooth, nonporous planar solids, contact angle measurements can be easily accomplished by placing a droplet of liquid such as carbon tetrachloride on the surface of the substrate and measuring the angle by using a microscope fitted with a goniometer eyepiece.

For the polypropylene filters, however, contact angle measurements is a little more difficult. First, carbon tetrachloride should not be used because it is too hydrophobic and is absorbed into the fibers upon contact. A preferable liquid that is suitable for this material is 1,1,2,2-tetrabromoethane. Secondly, the surface of a microporous filter is not smooth. Surface roughness can increase or decrease the contact angle. For wetting surfaces,  $\theta < 90^\circ$ , surface roughness reduces the contact angle, but increases for non-wetting surfaces (Davies *et al.*, 1961). For this reason, an average of six readings were taken at different locations on the filter surface. High contact angle can indicate that the surface is hydrophilic. Conversely, low contact angle can mean that the surface is hydrophobic. The average contact angle for the untreated filters was approximately  $25^\circ$ . The 5 mM DDAB solution treated filters had the lowest contact angles among the treated filters, and the 10 mM DDAB treated filters had the highest contact angles.

Since the standard deviation of these measurement were very large, the difference in 10 and 20 mM DDAB treated filters were not very significant. Therefore, the 10 mM treatment was chosen as the standard method of treatment for the filters.

The adsorption strength of the cationic surfactant was tested by flushing the treated  
5 filters with different amounts of water and then measuring the contact angles. The amount of water pumped through the filters did not have a significant effect on the contact angle, which indicated that the surfactant was not desorbing. The effluents were collected for surface tension measurements. The results indicated that the surfactant was not desorbing as detected by surface tension measurements. From previous experiments, it has been shown that  $2.5 \times 10^{-5}$  moles/L of  
10 the surfactant in water decreased the surface tension of water from 72.4 to 43.9 dynes/cm. Therefore, surface tension method is able to detect DDAB in the range of micromoles per liter of water.

Since both the filters and the particles are nonconductive, they were coated with gold before scanning electron micrographs were taken. Due to the limitation of the method and  
15 availability of the particles, adsorption of particles of larger sizes (197 nm and 200 nm) than those used in the actual filtration experiments (60 nm) were studied. The zeta potential of the untreated filters indicated that the filters were slightly negatively charged. The untreated and 10 mM DDAB treated AN06 filters were made by a melt-blown process and were fibrous. Since the untreated filters are slightly negatively charged, negatively charged particles will not adsorb  
20 on the surface due to the electrostatic repulsion, but positively charged particles will adsorb due to the electrostatic attraction. Experimental results confirm this. Only a few negatively charged particles attached to the surface of the untreated filter, whereas significantly more positively charged particles attached. The zeta potential of the surfactant treated filters exhibited a positive charge for the whole pH range. In this case, a negligible number of the positively charged  
25 particles adsorbed on the surface. The negatively charged particles, however, were overwhelmingly attracted to the surface. Particle adsorption is also a function of the suspension concentration. By increasing the suspension concentration, more particles will diffuse to the surface of the filter and adsorb on the surface. Effects of concentrations from 0.020 wt% solids to 0.050 wt% solids show that the number of particles adsorbed on the filter surface increases  
30 with their concentration in the aqueous phase.

Filtration efficiency or percent removal was significantly enhanced with DDAB treatment. For the untreated filters, the filtration efficiency ranged from 5% to 10%, but after the filters were treated with 10 mM DDAB, filtration efficiency increased to 50% or 60% for the lower initial concentration range. The increase in capture efficiency was mainly due to the  
35 electrostatic attraction between the negatively charged particles and the positively charged polar

head of the surfactant molecules on the filter surface. Since the average pore size was 0.6  $\mu\text{m}$ , the increase in capture efficiency was partly due to the smaller pore size in certain regions of the filters due to surfactant adsorption in clusters. However, by examining the results for the filtration at pH 10.0, it is evident that the increase due to entrapment was not very significant since the filtration efficiency of the treated and untreated filters were very similar. If entrapment is the main mechanism, then filtration efficiency would not be dependent on the pH of the suspension unless pH induced flocculation was occurring. However, if pH induced flocculation was taking place, then the capture efficiency of the untreated filters would have increased as pH was lowered. This was not observed experimentally. In addition, quasi-elastic light scattering measurements showed that the average particle size did not increase with decreasing suspension pH, indicating an absence of flocculation at lower pH.

Since the particles are more negatively charged at higher pH values due to a higher degree of ionization, higher filtration efficiency was expected at the higher pH range. However, the results showed the opposite trend. Capture efficiency was higher at the lower pH range. This trend could have been due to one of the following two effects. Either the surfactant was desorbing at the higher pH range or competitive ion adsorption was taking place.

To determine if the surfactant was desorbing, the treated filters were flushed with pH 10.0 solution. The effluents were collected for surface tension measurements. If the DDAB was desorbing, then surface tension of the effluents should have been lower than the pure water due to the surfactants at the surface. Results indicated that there was no change in surface tension of the effluents. Further proof that the surfactants were not desorbing was offered by contact angle measurements. Even with 200 ml of pH 10.0 solution flush, the average contact angle of the surface,  $70.7 \pm 12.7$  degrees, remained approximately the same as those that were flushed with 30 ml distilled water. Finally, the filters were rinsed with 30 ml of pH 10.0 solution, followed by 20 ml of distilled water, and then used to filter particles at pH 4.0. Results indicated that the filters were just as effective as those that were rinsed with distilled water, which is another indication that the surfactant was not desorbing at the higher pH values.

Since the surfactants were not desorbing, the only other explanation was that the hydroxide ions were competing with the negatively charged particles for the positively charged surfactant sites. At pH 10.0, for examples, because of the high number of hydroxide ions and because of their higher mobility due to their smaller size, they diffused to the surface of the filter much faster than the large negatively charged particles. It is believed that there was the mechanism of competitive adsorption of low and high suspension pH values, where the adsorption of the hydroxide ions shielded the charge of the surfactants from the particles, and therefore, the particles were not adsorbing to the surface of the filters.

Example 2 — Novel Method for Coating Filter Media with Precipitates of Metallic Hydroxides and/or Metallic Oxides and the Resulting Coated Filter Media

The subject invention pertains to a novel method for coating filter media with precipitates of metallic hydroxides and/or metallic oxides and the resulting coated filter media.

5 The resulting coated filter media can be utilized in, for example, survival or personal water purification devices, household water filters, air filters, water reclamation filter media, water remediation processes, soil remediation processes, viral removal filters, and protective clothing.

Fiberglass air filters, woven and nonwoven filters or fabrics, sand, and diatomaceous earth were coated in this experiment, as already discussed above. Their removal efficiencies  
10 were determined for the individual contaminants studied.

Example 3 — Filtration of Microbes with Surfactant Treated Polypropylene Filters

This example pertains to the use of surfactant treated polypropylene filters for filtration of bacteria and bacteriophages. Two different types of bacteria and three different types of  
15 bacteriophages were chosen for experiments to determine the effectiveness of such filtration. The two bacteria were *Staphylococcus aureus* (ATCC 12600), a gram-positive bacterium, and *Escherichia coli* (ATCC 15597), a gram-negative bacterium. Bacteriophages were used as surrogates for human pathogens. Three well-studied phages were chosen to represent a wide range of properties that human pathogens may have. The phages and their respective hosts were  
20 MS2 (*Escherichia coli* C-3000),  $\Phi$ X-174 (*Escherichia coli* KC), and PRD-1 (*Salmonella typhimurium*). A comparison between MS2 and PRD-1 can assist to delineate the effect of size of viruses and that between MS2 and  $\Phi$ X-174 can help reveal the effect of isoelectric point or charge characteristics. All of the filtration efficiency and filter coefficient calculations are based on the number of viable bacteria and bacteriophages. Therefore, it must be pointed out that  
25 although the term "adsorption" or "adhesion" is used to account for the differences between the number of bacteria in the influent and the effluent, some of these differences may be due to the killing of the bacteria or bacteriophages by the surface coatings or by the shearing or transport process. The filter coefficient, in essence, accounts for all mechanisms of microbial reduction in the solution. Throughout this application the terms "bacteriophage", "phage", and "virus" will  
30 be used interchangeably.

The microporous polypropylene filters can be treated as earlier described in Example 1. For the filtration of bacteria and phages, AN25 filters, which has mean pore diameter of 2.5  $\mu$ m, were used. By using larger pore size filters, pressure drop can be decreased and leakage through the filter holders minimized. In the filtration of microbial, multiple layers of filters can  
35 be used. The bacteria used for the experiments were grown overnight in a 3% tryptic soy broth

and then diluted by a factor of 1000 in a buffer made of 0.02 M imidazole and 0.02 M glycine and adjusted with HCl to pH 7.0. This gives approximate initial concentrations of  $2 \times 10^5$  for *S. aureus* and  $1 \times 10^5$  for *E. Coli*.

The filtration apparatus consisted of a variable speed Infusion-Withdrawal Syringe Pump (Harvard Apparatus Co., MA), six 25 mm stainless steel filter holders (Fisher Scientific) connected in series, and plastic valves and connectors for sampling ports. Each filter holder contained 1 layer of the polypropylene filter. The filters were oriented vertically and the flow direction was upward so that no air bubbles would be trapped in the filters or in the line. The flowrate was kept a constant flow of 10 ml/minute.

A total of 120 ml of contaminated water was pumped through the filters. After approximately 20 ml of the contaminated water had passed through the filters, the first sample was taken starting from port 1 on the bottom. After all the samples from the different ports were taken, a second sample was taken from each port in the reverse order, and then a third sample was taken from each port starting from the bottom port. The samples were diluted with a solution containing 1% tryptic soy broth. Bacterial concentration was measured within 12 hours by the spread plate method (Gerhardt, 1994) using Mannitol Salt Agar for *E. aureus* and MacConkey Agar for *E. Coli*. Each sample was plated in duplicates.

The same apparatus and procedures were used in the bacteriophage filtration experiments. Initial concentrations of the phages were approximately  $3 \times 10^5$ ,  $3 \times 10^6$  and  $6 \times 10^4$  PFU/ml for MS2, PRD-1 and  $\Phi$ X-174, respectively. The phages were assayed immediately after filtration using the soft agar overlay method described by Snudstad and Dean (1971).

In the filtration of bacteria, *S. aureus* and *E. coli* were mixed together in one solution before they were used in the experiment. Similarly, in the filtration of bacteriophages, all three phages were added to the buffer solution at one time. This was done to reduce the number of experiments required. Since both bacteria were tested at the same time, the preferential adsorption of the bacteria can also be determined from the results. Similarly, the preferential adsorption of the phages can be observed as well. Information on the selective adsorption of one microbe over the others is important because in real life situations, the contaminated water always contains more than one type of microorganism.

The untreated polypropylene filter is negatively charged in the range of pH 4.0 to 10.0, where the 10-mM DDAB treated filter, on the other hand, is positively charged in the same pH range. Results of the filtration of both *E. coli* and *S. aureus* are shown. The 10 mM surfactant treated filters had much better bacterial removal than the untreated filters. The data points are fitted with the following equation in order to obtain the filter coefficient:

$$\eta = \frac{C_{in} - C_{out}}{C_{in}} = 1 - e^{-\lambda L} \quad (3-1)$$

where  $\eta$  is the filtration efficiency or percent removal,  $C_{in}$  is the initial concentration in colony forming units per milliliter (CFU/ml) for bacteria and plaque forming units (PFU/ml) for phages,  $C_{out}$  is the final concentration,  $L$  is the length of the filter in units of number of layers, and  $\lambda$  is the filter coefficient with units of 1/layer of filter. Converting the results into filter coefficients is desirable for comparing the effectiveness of the different coatings for bacterial removal. The filter coefficients and coefficients of linear regression are shown in Table 3-2. The coefficient of linear regression indicates how well the data points are fitted to the filtration equation, with a value of 1.0 being a perfect fit. The filter coefficients of the untreated filters are 0.485 and 0.353 [1/number of filters] in the filtration of *S. aureus* and *E. Coli*, respectively. The filter coefficients of the 10 mM DDAB treated filters are 1.039 and 0.896 in the filtration of *S. aureus* and *E. Coli*, respectively. From the filtration equation, it is obvious that the higher the filter coefficient, the better the filtration efficiency. The filter coefficients as defined in this application have units of 1/number of filters. In the filtration of *S. aureus*, there is a 114% improvement in the filter coefficient. In other words, to obtain the same percent removal of *S. aureus*, the untreated filters would require 114% more layers of filters than the surfactant treated filters. For the filtration of *E. coli*, the improvement in filtration coefficient is even more significant than the improvement for *S. aureus*. The improvement for *E. coli* filtration was 154%.

	Table 3-2. Filter coefficient ( $\lambda$ ) and coefficient of linear regression ( $R^2$ ) for untreated and 10 mM DDAB treated AN25 for different bacteria.			
	<i>S. aureus</i>		<i>E. coli</i>	
	$\lambda$	$R^2$	$\lambda$	$R^2$
Untreated Filter	0.485	0.984	0.353	0.958
10 mM DDAB Treated Filter	1.039	0.996	0.896	0.984
Improvement Upon Coating	114%	---	154%	---

Since bacteriophages are much smaller than bacteria, the filtration efficiency or percent removal of both the untreated and surfactant treated filters are not expected to be as high as the

percent removal for the bacteria. The results for both the untreated and treated filters have a high degree of scattering or standard deviation. The data for the untreated filters have a very poor fit,  $R^2$  of 0.28 to 0.38, when fitted to Equation 3-1. The isoelectric points (IEP), the pH at which the zeta potential is zero, of bacteriophage MS2, PRD-1 and  $\Phi$ X-174 are 3.9, 4.2 and 6.6, respectively. At pH values below the isoelectric points, the phages are positively charged and at pH above the isoelectric points, they are negatively charged.

Although no zeta potential as a function of pH data are available, it can be inferred from the isoelectric point that in the neutral pH range, MS2 most likely has a higher negative charge density than PRD-1 and  $\Phi$ X-174. With an IEP of 6.6,  $\Phi$ X-174 is only slightly negatively charged at the experimental pH of 7.0. The positively charged surfactant coating on the polypropylene filters greatly improved the filtration efficiency of the filters for bacteriophages MS2 and PRD-1, but it was not as effective for the removal of bacteriophage  $\Phi$ X-174. The filter coefficients for the untreated polypropylene filters are 0.062, 0.069 and 0.069 for the filtration of MS2, PRD-1 and  $\Phi$ X-174, respectively. As expected the filter coefficients are very low because the phages are much smaller than the bacteria. In addition, the filter coefficients for the filtration of the three different phages for the untreated filters are the same because removal is negligible and probably due to straining or entrapment mechanism.

For the surfactant treated filters, the filter coefficients are 0.345, 0.269 and 0.084 for the filtration of MS2, PRD-1 and  $\Phi$ X-174, respectively. In the filtration of MS2, there is an improvement of 458% in the filter coefficient. In the filtration of PRD-1, there is an improvement of 288% in filter coefficient, and in the filtration of phage  $\Phi$ X-174 a 22% improvement is obtained. This small improvement is within the standard deviation and therefore, surface modifications based on Coulombic interaction may not be helpful in the filtration of phage  $\Phi$ X-174. This is possibly due to the fact that the phage is very close to its isoelectric point (i.e. pH 6.6). Bacteriophage MS2 has the lowest isoelectric point and the highest filter coefficient. These results reinforced the idea that electrostatic attraction is an important factor in bacterial and viral adsorption to surfaces.

Table 3-3. Filter coefficient and coefficient of linear regression for untreated and 10 mM DDAB treated AN25 for different bacteriophages.						
	MS2		PRD-1		ΦX-174	
	$\lambda$	R <sup>2</sup>	$\lambda$	R <sup>2</sup>	$\lambda$	R <sup>2</sup>
Untreated	0.062	0.279	0.069	0.357	0.069	0.374
Treated	0.345	0.920	0.269	0.913	0.084	0.602
Improvement	458%	---	288%	---	22%	---

Since the concentrations used in the experiments were too low for the bacteria to be seen on the filter surface under higher magnification, the filters were soaked in a solution containing high concentrations ( $1 \times 10^7$  CFU/ml) of both *E. coli* and *S. aureus* for 30 minutes. After soaking in the solution for 30 minutes, the filters were thoroughly washed three times with deionized water. They were then placed in a container containing osmium tetroxide vapor for two days to fix the cells so that they would not burst, rupture or shrink in the drying stage. The filters were then air-dried and coated with gold for SEM analysis. By looking at the wettability of the filters, it appeared that the filter wettability was not uniform throughout the whole filter.

Bacterial adsorption on the filter surface was not uniform throughout the filter surface. Studying *S. aureus* and *E. coli* adsorption on the 10 mM DDAB treated polypropylene filter showed most of the microbial adsorption occurred in the center region of the filter. Areas around the edges have some bacteria but not as high a concentration as the center region. For the untreated filter, only a few *S. aureus* and *E. coli* cells were found on the filter surface. The surfactant treated filter, on the other hand, attracted much more bacteria to the surface of the filter, especially in localized areas. Significant difference existed between the adsorption of bacteria on the untreated and surfactant treated filters. The spherical particles are *S. aureus* bacterium, and the rod-like particles are *E. coli* bacterium.

Example 4 – Surfactant Treated Microdenier Polyester

Polyester yarn has many consumer and industrial uses, including clothing, tire cord thread, conveying belts, waterproof canvas, and fishing nets. Microdenier polyester textile fabric was chosen for the filtration of nanoparticles and biological particles because it is readily available, and it is tenacious. Therefore, it can handle high flowrates and high pressure drops. In addition, because it is textured and woven, it increases the tortuosity of the flow path and therefore increases the probability that particles will collide with the surface of the filters. The yarn used to make the fabric, as described in the next section, is a microdenier yarn, but the term "microdenier polyester" and "polyester" will be used interchangeably throughout the discussion of experimental result in this application since only one type of yarn was tested. Other terms, which will have the same meaning in this context are bacteriophages, phages and viruses. Bacteriophages or phages are host-specific viruses. Therefore, they will be used interchangeably.

The microdenier polyester fabric used in the subject experiments consists of a 2 x 2 right-hand twill, weighing approximately 5.5 ounces per square yard and constructed from a microdenier 1/140/200 textured polyester yarn, Type 56T Dacron® from DuPont in the warp direction and a microdenier 1/150/100 textured polyester yarn, Type 56T Dacron® from DuPont, in the filling direction. The fabric construction was 76 picks in the filling direction and 176 ends in the warp direction. The fabric was cut into 25-mm circles with a die cutter before the surfactant treatment was applied. Once the filters were cut into circles, they were treated with a 10 mM DDAB solution using procedures described earlier in.

Before the filters were used in the experiments, they were flushed with 120 ml of deionized water. The filtration apparatus used for these experiments are the same as described and shown in Example 3. The sampling and filtration procedures are the same as those used for Example 3 with the exception that each filter holder contained four layers of these fabric filters instead of one layer.

The zeta potentials of the untreated and 10 mM DDAB treated microdenier polyester filters were measured with a streaming potential apparatus. Six layers of the fabric were used in each measurement. The untreated filters are negatively charged from pH 4 to pH 9.0 while the surfactant treated polyester filters are positively charged in the same pH range.

For the pressure drop experiment, the apparatus is the same as that for the filtration experiments. The flowrate was kept at a constant rate of 10 ml/minute and the pressure at each port was measured with a digital pressure gauge. From these pressures, the pressure drop for each filter holder (4 layers of fabric filters) was calculated.

For the untreated filters the standard deviations are larger than the surfactant treated filters. For the untreated filters, the pressure drops ranges from 0.7 psi for 4 layers of filters to

3.1 psi for 24 layers of filters. For the surfactant treated filters, however, the pressure drop is much lower due to the increase in wettability of the textile fabric. The pressure drops of the surfactant treated filters are 0.2 psi and 1.1 psi for 4 layers and 24 layers of the fabric filters, respectively. The decrease in pressure drop with the surfactant treatment is beneficial because it will require less energy to pass the liquid through the filters. Essentially, for the same pressure drop, more throughputs can be achieved.

Two different types of bacteria were used in these experiments. The gram-positive bacterium was *E. coli* and the gram-negative was *S. aureus*. These bacteria were prepared according to the procedures described in Example 3. Filtration was done at pH 7.0 using a 0.02 M imidazole-glycine buffer. The initial concentrations of the bacteria were approximately  $1 \times 10^5$  CFU/ml for both bacteria. Flowrate was maintained at a constant rate of 10 ml/minute with the use of a syringe pump. Samples were collected at three separate times and each sample was plated in duplicate. In general, the standard deviations of the untreated filters are larger than those of the surfactant treated filters. Also, the results of the first four layers always has a higher degree of scattering when compared to the rest of the filters. This is a common problem in filtration experiments, probably due to the fact that the flow has not been fully developed at the entrance of the set-up.

Results show that the untreated polyester fabric filters removed more *S. aureus* than *E. coli*. One possible explanation is that the *E. coli* is more negatively charged than the *S. aureus* (Sonohara *et al.*, 1995), and therefore the repulsion between the filter surface and the bacterium is greater. In their study of bacterial adhesion to different fabrics, Hsieh and Merry (1986) also found that *S. aureus* has a stronger affinity for [untreated] polyester than *E. coli*. The surfactant treated polyester filter, on the other hand, removed the same percentage of both bacteria from the aqueous streams. It has been shown that for small particles (Spielman and Friedlander, 1973; Ruckenstein and Prieve, 1973; Li and Park, 1997), the process is diffusion limited once the repulsive energy barrier is removed. In this case since the surface is positively charged and the bacteria are negatively charged, there does not appear to be any significant barrier to the adsorption or adhesion process. The rate of bacterial adsorption/adhesion to the filter surface is likely limited by the transport of the bacteria to the surface and/or the collision of the bacteria with the filter surface. Therefore, since the bacteria are of approximately the same size, it is expected that the removal would be similar for *E. coli* and *S. aureus*, which was observed experimentally.

Table 4.1. Filter coefficient and coefficient of linear regression for untreated and 10 mM DDAB treated polyester fabric filter for different bacteria.				
	<i>S. aureus</i>		<i>E. coli</i>	
	$\lambda$	$R^2$	$\lambda$	$R^2$
Untreated Filter	0.085	0.966	0.055	0.984
10 mM DDAB Treated Filter	0.251	0.998	0.238	0.994
Improvement	195%	---	330%	---

The filter coefficients of the untreated polyester fabric filters are 0.085 and 0.055 [1/filter] for the filtration of *S. aureus* and *E. coli*, respectively. For the untreated filters, the process is not transport or diffusion limited because of the repulsive barrier between the bacteria and the filter surface. Since there is a difference in the filter coefficient or filter efficiency of the untreated filters, factors other than Coulombic interaction are probably involved. It is possible that hydrophobic or other specific interactions are involved. For the DDAB treated filters, the filter coefficients are 0.251 and 0.238 for the filtration of *S. aureus* and *E. coli*, respectively. Within experimental errors, these two numbers can be considered to be identical. The surfactant treatment improved the filter coefficient by 195% for the filtration of *S. aureus* and 330% for the filtration of *E. coli*.

The same apparatus and procedures were used in the bacteriophage filtration experiments. Initial concentration of the phages were approximately  $3 \times 10^5$ ,  $6 \times 10^4$  and  $3 \times 10^6$  PFU/ml for MS2,  $\Phi$ X-174 and PRD-1, respectively. The phages were assayed immediately after filtration using the soft agar overlay method described by Snudstad and Dean (1971).

For the filtration of MS2, only approximately 20% of the phage in the influent could be removed with 24 layers of the untreated polyester filter. These removal efficiencies are much lower than those of the bacteria because the phages are roughly 10 times smaller than the bacteria. The surfactant treatment significantly increased the filtration efficiency of MS2. With 24 layers of the treated filters, over 98% of the MS2 in the influent were removed. Similar trends were observed for the filtration of PRD-1, with 24 layers of treated filters removing over 97% of the phage in the influent. In the filtration of bacteriophage  $\Phi$ X-174, the results are very different. The surfactant treated filters were not as effective at removing  $\Phi$ X-174 as they were at removing MS2 and PRD-1. Even with 24 layers of filters, less than 40% of the influent phage was removed.

All of the data points were fitted to an exponential decay equation described in Example 3. The filter coefficient of the untreated filters for the filtration of MS2, PRD-1 and  $\Phi$ X-174

are 0.008, 0.005 and 0.009, respectively. Because of the large scattering of the data for the untreated filters, the coefficients are statistically the same. The viruses or phages are not expected to adsorb to the surface of the untreated filters. Therefore, the removal is likely due to straining or entrapment of the particles in the structure of the filters. For the surfactant treated filters, however, the results of the different phages are very different. The filter coefficients are 0.268, 0.158 and 0.018 for the filtration of MS2, PRD-1 and  $\Phi$ X-174, respectively. There is a strong correlation between the isoelectric point (IEP), the pH at which the zeta potential is zero, and the filter coefficients, and hence the filtration efficiency. The isoelectric point of bacteriophage MS2, PRD-1 and  $\Phi$ X-174 are 3.9, 4.2 and 6.6, respectively. Looking at these numbers, MS2 has the lowest isoelectric point, followed by PRD- 1 and then  $\Phi$ X174.

Because of the low IEP, MS2 should be the most negatively charged ph age at the experiment condition, follow by PRD- 1 and  $\Phi$ X- 174. Due to this high charge density, MS2 has the highest adsorption to the filter. The surfactant treatment increases the filter coefficient by 3300%, which means that to obtain the same percentage removal of MS2, the length (or number of filter in this case) of the untreated filters must be 33 times longer than the treated filters. The filter coefficient improves by 2800% in the filtration of phage PRD-1. In the filtration of  $\Phi$ X-174, the filter coefficient improves by only 98%. This improvement is rather insignificant because the standard deviation is fairly high. Because of its high IEP of 6.6, the phage is only slightly negatively charged at the experimental pH of 7.0. Other researchers have shown that  $\Phi$ X-174 is one of the most difficult phage to remove from aqueous and air streams because of its poor adhesion to surfaces.

To verify that the bacteria were attracted to the surface of the filters, the first layer was examined with a scanning electron microscope after 1.0 L of the bacterial solution, containing both *S. aureus* (approximately  $1 \times 10^5$  CFU/ml) and *E. coli* (approximately  $1 \times 10^5$  CFU/ml), had passed through it. The filter was then rinsed with deionized water in a beaker to remove loose bacteria from the surface. The wet sample was prepared for SEM analysis by placing the contaminated filter in a chamber saturated with osmium tetroxide vapor for 2 days. The filter was then taken out and allowed to dry overnight.

Once the sample was dry, it was coated with a thin layer of gold. SEM analysis was done with a Hitachi S4000 Field Emission Scanning Electron Microscope (Tokyo, Japan). The amount of bacteria on the surface of the filters is very small. These pictures are not representative of the surface of the filters. The bacterial concentration in these filtration studies are only approximately  $1 \times 10^5$  CPU/ml. Therefore, it is not expected that many bacteria would be found at such a small surface area, due to the magnification required to see the microorganisms. It is also possible that many of the bacteria did not survive the SEM

preparation procedure. Some of the cells might have died and ruptured before they could be fixed with osmium tetroxide vapor.

In an effort to show that the surfaces of the treated filters are truly more active than the untreated filter, solutions containing higher concentration of bacteria were used. In this study, each filter was soaked in solution containing 2.0 ml of *S. aureus* and *E. coli* mixture for 30 minutes. The bacteria solutions were taken directly from the growth broth without dilution. Their concentrations were approximately  $1 \times 10^7$  CPU/ml. After soaking for 30 minutes, the filters were rinsed with deionized water three times before they were fixed with osmium tetroxide vapor and analyzed according to the previous described procedure. Bacterial (*S. aureus* and *E. coli*) adhesion to the surface of the untreated polyester fabric filter is shown in Figure 4-12. Only a few bacteria are seen in these pictures. The untreated filters are negatively charged and, therefore, may actually repel the bacteria rather attract them. The surfactant treated filters, on the other hand, are positively charged and, therefore, the bacteria are likely attracted to the filter surface. Significantly more bacteria, *S. aureus* and *E. coli*, adsorbed to the treated filter surface. It appears that surface charge modification can substantially increase the number of bioparticles adhering to the filter surface, and hence increase the filtration efficiency.

Example 5 – Removal of Microorganisms from Water by Sand Coated with Ferric and Aluminum Hydroxides.

The source of sand was "All Purpose Sand" from Pebble Junction, Division of Delaware Valley Landscape Stone, Inc., Sanford, Florida. Sand was sieved to a size less than 100 mesh before use. For coating, the sand was soaked in one of the following solutions: aluminum chloride or ferric chloride (Fisher Scientific, Pittsburgh, PA) at concentrations of 0.05 M, 0.1 M, 0.5 M, or 1.0 M. In some tests a combination of 1.0 M ferric chloride and 1.0 aluminum chloride was used. The coating was applied with heat and agitation similar to temperatures and agitation described in Examples 2 and 6. Sufficient solution was used to completely cover the sand. After 30 minutes, the solution was poured off and the sand was allowed to dry. Occasional stirring was used to break up the clumps that formed and ensure that the sand was thoroughly dry. Next, the sand was added to 2 volumes of 3 M ammonium hydroxide and was allowed to soak for 10 minutes. The ammonium hydroxide was poured off, the sand was dried again with occasional stirring. The dried sand was rinsed and stored at room temperature until used. *Escherichia coli* (ATCC 13607) was obtained from the American Type Culture Collection and was used in batch and column adsorption experiments. *Escherichia coli* was routinely grown in Tryptic Soy Broth (Difco Labs, Detroit, MI) and assayed using MacConkey Agar (Difco Labs, Detroit, MI). A *Vibrio cholera* strain 124 isolated from Chillon River, Lima,

Peru, was supplied by Dr. Tamplin of Department of Food Science at the University of Florida. It was grown in Tryptic Soy Broth and assayed using Tryptic Soy Agar (Difco Labs, Detroit, MI) plates containing 1% sodium chloride (pH 7).

Poliovirus 1 (strain LSc-2ab) was grown on buffalo green monkey (BGM) cells and  
5 assayed as plaque forming units (PFU) by using an agar overlay technique (Smith and Gerba, 1982). Bacteriophage MS-2 was grown on *Escherichia coli* C-3000 (ATCC 15597) and assayed by soft agar overlay (Snustad and Dean, 1971).

All the tap water used in this study was dechlorinated by the addition of sodium thiosulfate, residual chlorine was determined using O-tolidine (American Public Health  
10 Association, 1995). The dechlorinated tap water was then seeded with bacteria or viruses. Raw sewage was collected from the University of Florida sewage treatment plant and used on the same day.

Modified or untreated sand was packed in columns of different sizes. The sizes of columns used and the weight of dry sand added were as followed: A. Small: 10 X 2.5 cm with  
15 80g sand; B: Large: 35 X 5 cm, with 1kg sand. Glasswool (Fisher Scientific, Pittsburgh, PA) was placed in the bottom of each columns. Before being used in experiments, deionized water was passed through the columns till the unbound metallic hydroxide was removed and the column effluents were clear and free of precipitates.

Samples were passed through the columns by using gravity flow (small-sized columns)  
20 or under a positive pressure by pressurized nitrogen gas (large-sized columns). In small-sized columns, 160 ml of samples were separately passed through the columns. Samples of 40 ml were collected and assayed. The initial samples, the column effluents, and the rinse were assayed to determine the removal efficiency. In the large-sized columns, four liters of sample was passed through the columns at 450 ml/min. After the passage of the first 500 ml, we  
25 assayed the column effluents. Both the initial samples and the column effluents, were assayed for bacteria or viruses.

Four liters of tap water were dechlorinated by the addition of sodium thiosulfate (0.0002% final concentration) and was passed through columns (35.0 X 5.0 cm) containing one kilogram of modified (with 1.0 M aluminum chloride and 1.0 M ferric chloride) or unmodified  
30 sand at a rate of 450 ml./min. daily for 48 days. Once a week, 4 liters of dechlorinated tap water seeded with *E. coli* KC, and MS-2, was passed through the columns at the same flow rate and collected. Initially and after the passage of 120 liters of water through the filters (29 days), the columns were challenged with polio virus 1 in addition to the other two microorganisms. This was repeated after the passage of 198 L of water (48 days). Both the initial samples and

the column effluents were assayed for the three microorganisms. By the end of the study, 192 liters of water had been passed through each of the sand columns.

After adsorption experiments with small columns (10 X 2.5 cm, 80 g) were conducted as described above, 40 ml of rinse water was passed through the column and collected, then 160  
5 ml of 3% beef extract at pH 7 (Becton Dickinson, Cockeysville, MD) was passed to elute adsorbed microorganisms. Microorganisms in the initial sample, the column effluent, the rinse, and the column eluate were assayed for bacteria or viruses to determine the removal and recovery efficiencies.

Tap water dechlorinated by sodium thiosulfate was passed through columns containing  
10 modified or untreated sand columns (10 X 2.5 cm, 80 g). The column effluents and a tap water control were seeded with microorganisms. The samples were assayed initially and after 3 hours incubation at room temperature to determine the effect of the column effluents on survival of the microorganisms.

Modified or unmodified sand (5 g) was mixed with 7 ml of one of each of the following  
15 buffers that had been seeded with *E. coli* or MS-2: A: 0.02M imidazole and 0.02M glycine, pH 7; B: Buffer A + 0.1M sodium citrate, pH 7; C: Buffer A + 0.1% teen 80, pH 7; D: Buffer A + 0.1M sodium citrate, 0.1% teen 80, pH 7). The samples were mixed on a rotary shaker at approximately 100 rpm for 15 minutes. The initial and final supernatant were assayed. Microorganisms were also added to the buffer samples without sand as controls.

20 Modified or unmodified sand (5 g) was shaken with 7 ml of buffer (0.02M imidazole and 0.02M glycine, pH 7) that had been seeded with *E. coli* or MS-2 at approximately 100 rpm for 15 minutes. After this period of adsorption, the supernatant was removed and assayed. Next, 7 ml of one of each of the following buffers was added: A: 0.02M imidazole and 0.02M glycine, pH 7; B: Buffer A + 0.1M sodium citrate, pH 7; C: Buffer A + 0.1% teen 80, pH 7;  
25 D: Buffer A + 0.1M sodium citrate, 0.1% teen 80, pH 7; E: 3% beef extract, pH 7). The samples were shaken for another 15 minutes at 100 rpm on a New Brunswick Scientific orbital shaker. The initial seeded buffer, the supernatant sample from the adsorption step, and the supernatant samples from the elution steps were assayed. As controls, the buffer samples without sand were seeded with *E. coli* and MS-2 and shaken for 15 minutes to observe their  
30 effects on the test organisms.

Standard deviations, slopes, correlation and general t test probabilities were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah).

Column influents and effluents were analyzed by Inductive Coupled Argon Plasma Spectroscopy.

The effect of different concentrations of ferric and aluminum chloride, used for modifying the sand, on the adsorption of microorganisms to sand columns (10 x 2.5 cm., 80 g. sand) are shown in Table 5-1. The higher concentration of chemicals added for modifying the sand, the greater the removal of microorganisms. There was a linear correlation between the concentration of the sal used to treat the sand and the  $\log_{10}$  removal of the microorganisms tested,  $r > 0.8$ . Columns containing sand modified by using 0.1M of  $\text{FeCl}_3$  reduced both *E. coli* and MS-2 by greater than 99.9%. Equivalent removals were obtained using sand modified with 0.05M aluminum chloride. Columns containing sand modified with higher concentrations of metal chloride reduced the concentrations of *E. coli* and MS-2 by greater than 5  $\log_{10}$ . In contrast, columns containing untreated sand only removed 0.53  $\log_{10}$  of MS-2 and less than 0.62  $\log_{10}$  of *Escherichia coli*. The flow rate of both the treated and untreated sand columns were about 90 ml/min. The pressure drop in across the treated and untreated sand columns was similar (data not shown).

Columns (10 x 2.5 cm., 80 g. sand) containing sand modified using 0.1 M ferric chloride and 0.1 M aluminum chloride reduced MS-2, *Escherichia coli*, and *Vibrio cholera* by 7.7  $\log_{10}$ , 5.3  $\log_{10}$ , and 2.2  $\log_{10}$ , respectively (Table 5-2). The combination of both chemicals for modifying sand used in filters had a synergistic effect. Increasing the concentration of both salts used for modifying the sand to 1.0 M produced little or no change in removal of MS-2 but did significantly increase the removal of *E. coli* and *V. cholera*. Sand modified with the combination of 1 M ferric chloride and 1 M aluminum chloride was used in additional studies on removal of microorganisms from water.

The large columns with the modified sand reduced the number of *E. coli* by 5  $\text{Log}_{10}$  (99.999% removal) after passage of 28 liters of water. After the passage of 172 liters of tap water, the columns removed more than 90% of *E. coli*. However, with the passage of more water, the columns with modified sand showed a decreased ability to remove *E. coli*. At the end of the test, columns containing either treated or untreated sand were similar in their ability to remove *E. coli*. In contrast, columns containing modified sand reduced the number of MS-2 by more than 5  $\log_{10}$  after the passage of 148 liters tap water. After 192 liters of water were passed through the columns, they could still remove 99.9% MS-2. Columns containing modified sand could reduce the level of polio 1 by 4.1  $\log_{10}$  units at day 1. More than a 3  $\log_{10}$  reduction was obtained after the passage of 120 liters of water, while columns containing untreated sand removed less than 1  $\log_{10}$  of polio 1 from the seeded tap water throughout the experiment. After the passage of 192 liters either columns removed approximately 90% of polio 1 from tap water.

Raw sewage seeded with MS-2 was passed through the columns (10.0 x 2.5 cm, 80 g) at different temperatures. The modified sand columns reduced more than 4 log<sub>10</sub> of coliform bacteria from raw sewage at room temperature. The removal ability was related to temperature, as better removal was observed at higher temperatures. Columns containing modified sand removed more than a 3 log<sub>10</sub> of MS-2 at each of the three temperatures tested. More MS-2 was removed at 25°C and 37°C than at 4°C. . Untreated columns at all tested temperatures, had a log<sub>10</sub> removal of less than 0.3 and 1.0 for MS-2 and *E. Coli*, respectively.

In column adsorption-elution experiments, the modified sand columns removed more than 99.9% of both *E. coli* and MS-2 from water (Table 5-3). None of the microorganisms adsorbed to the modified sand columns could be detected in the dechlorinated tap water rinse. However, more than 10% of the retained microorganisms could be washed off from columns containing untreated sand. When 3% beef extract with 0.1M sodium citrate at pH 7 was passed through the columns, a higher percentage of microorganisms was eluted from both modified and untreated sand columns. However, in the case of *E. coli*, the total recovery from columns with modified sand was only about 5%. A higher percentage of *E. coli* was eluted of the columns containing unmodified sand even though these columns had a lower removal efficiency. It was possible to account for 74% of the *E. coli* that was initially applied to the columns. Most of the MS-2 (66%) adsorbed to columns containing modified sand could be eluted. It was possible to account for about 90% of the influent MS-2 in columns containing untreated sand. The effluents of columns containing the modified sand did not inactivate *E. coli* or MS-2 when seeded with them and incubated at room temperature for three hours (data not shown).

In batch adsorption experiments (Table 5-4), it was found that Teen 80 interfered with adsorption of *E. coli*, but had little effect on the adsorption of MS-2. Sodium citrate decreased the adsorption of *E. coli*, and completely eliminated the adsorption of MS-2. The combination of Teen 80 and sodium citrate reduced adsorption of *E. coli* and MS-2 about as much as citrate alone did.

Following adsorption of microorganisms in batch experiments, solutions were tested for their ability to elute the adsorbed microorganisms (Table 5-5). Solutions of buffer alone or buffer with Teen 80 did not elute any of the adsorbed microorganisms. A portion of the adsorbed *E. coli* and MS-2 was eluted by solutions of sodium citrate, sodium citrate + Teen 80, and beef extract. Neither the buffer nor the eluants inactivated MS-2 or *E. coli*, except for sodium citrate which inactivated 90% of the *E. coli* after three hours (data not shown). No inactivation was observed within the 20 min. duration of the test.

Columns containing modified sand removed more microorganisms than the columns containing untreated sand at each pH tested (pH 5, pH 7, and pH 9) (Table 5-6). The best removals were observed at pH 7 for both *E. coli* and MS-2.

Column effluents and influents contained less than 0.01 µg/ml ferric or aluminum when tested by the Inductively Coupled Argon Plasma Spectroscopy.

**Table 5-1 The effect of the concentration of ferric or aluminum salt used to modify the sand on the removal of microorganisms by modified sand columns\***

Salt Concentration (M/L)	Reduction (Log <sub>10</sub> ) Ferric Chloride Treated Sand		Reduction (Log <sub>10</sub> ) Aluminum Chloride-Treated Sand	
	MS-2	E. Coli	MS-2	E. Coli
0	0.53 ± 0.27	0.62 ± 0.28	0.53 ± 0.27	0.62 ± 0.28
0.05	0.20 ± 0.18	2.78 ± 0.25	3.60 ± 0.20	2.70 ± 0.20
0.10	2.70 ± 0.10	2.46 ± 0.20	4.80 ± 0.20	3.20 ± 0.30
0.50	4.50 ± 0.50	5.46 ± 0.10	5.55 ± 0.15	3.50 ± 0.5
1.00	5.30 ± 0.10	5.46 ± 0.10	6.65 ± 0.10	4.00 ± 0.10
Correlation (r)	0.89	0.87	0.80	0.85

\*Columns (10.0 x 2.5 cm) containing 80 g of 100 mesh sand modified with FeCl<sub>3</sub> and AlCl<sub>3</sub>. Values represent the average and standard deviation of triplicate runs.

**Table 5-2. The removal of microorganisms by filters containing said modified by a combination of FeCl<sub>3</sub> and AlCl<sub>3</sub>**

Microorganisms	Log <sub>10</sub> Reductions		
	Untreated	0.1M FeCl <sub>3</sub> + 0.1M AlCl <sub>3</sub>	1.0M FeCl <sub>3</sub> + 1.0M AlCl <sub>3</sub>
MS-2	0.3 ± 0.3 A	7.7 ± 0.0 B	7.7 ± 0.6 B
<i>Escherichia coli</i>	0.6 ± 0.1 A	5.3 ± 0.8 B	6.5 ± 0.0 C
<i>Vibrio cholera</i>	0.7 ± 0.5 A	2.2 ± 0.1 5.0 ± 0.0 C	5.0 ± 0.0 C

Notes: Values represent the mean and standard deviation for removal by three columns (10.0 x 2.5 cm, 80 g of 100 mesh sand). In each row, values with the same letter were not significantly different at P > 0.05.

5

Table 5-3. Elution of *Escherichia coli* and MS-2 adsorbed to sand filters.

Modifying Salt	Column Effluent (% of initial)		Tap Water Wash (% of initial)		Beef Extract (% of initial)		Total (% of initial)	
	<i>E. coli</i>	MS-2	<i>E. coli</i>	MS-2	<i>E. coli</i>	MS-2	<i>E. coli</i>	MS-2
1.0M FeCl <sub>3</sub>	< 1	< 1	< 1	< 1	5 ± 1	66 ± 8	5	66
None	36 ± 12	34 ± 6	10 ± 3	23 ± 6	28 ± 1	31 ± 1	74	90

10

Notes: An initial sample of 160 ml of dechlorinated tap water was seeded with approximately  $5 \times 10^6$  PFU/ml of MS-2 or  $1 \times 10^6$  PFU/ml of *Escherichia coli* and passed through 10.0 x 2.5 cm of columns containing 80 g of 100 mesh sand. Next, 40 ml of unseeded dechlorinated tap water was passed through the columns. Then, 160 ml of 3% beef extract + 1.0 M sodium citrate was passed through the columns. The percentages for MS-2 and *E. coli* in the column effluent, wash, and eluate were determined based on the number in the initial sample. Values represent the mean and standard deviation for triplicate columns.

15

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Table 5-4. Effect of salt and detergent on adsorption of *Escherichia coli* and MS2 to modified sand

Effect of the Solutions on	The Adsorption of <i>E. coli</i> (Log <sub>10</sub> Removal)	The Adsorption of MS-2 (Log <sub>10</sub> Removal)
Buffer	4.13 ± 0.72 A	5.23 ± 0.51 A
Buffer + 0.1M Sodium Citrate	1.64 ± 0.33 B	0.12 ± 0.18 B
Buffer + 0.1% Tween 80	1.18 ± 0.19 B	4.16 ± 0.45 A
Buffer + 0.1M Sodium Citrate + Tween 80	1.25 ± 0.17 B	0.58 ± 0.51 B

25

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Adsorption were conducted in the presence of the buffer that contained  $5.93 \pm 0.15 \log_{10}$  cfu/ml *E. coli* and  $5.28 \pm 0.48 \log_{10}$  pfu/ml. MS-2. Buffers: 0.02 M imidazole and 0.02 M glycine, pH 7; all the chemical solutions were made in this buffer and were adjusted to pH 7. Values represent the mean and standard deviation for triplicate determinations (5 g. 100 mesh sand treated with 1M FeCl<sub>3</sub> + 1M AlCl<sub>3</sub>). For each column, values followed by the same letter were not significantly different then  $P > 0.05$  level.

Table 5-5. Effect of salt and detergent on the elution of previously adsorbed MS-2 and *E. coli* from modified sand.

Effect of the Solutions on:	Elution of Previously Adsorbed <i>E. coli</i> (Log <sub>10</sub> bacteria eluted)	Elution of Previously Adsorbed MS-2 (Log <sub>10</sub> phages eluted)
Buffer	0.10 ± 0.10 B	0.10 ± 0.02 B
Buffer + 0.1M Sodium Citrate	2.65 ± 0.53 A	2.27 ± 0.41 A
Buffer + 0.1% Tween 8-	0.20 ± 0.10 B	0.08 ± 0.05 B
Buffer + 0.1M Sodium citrate + Tween 80	2.50 ± 0.23 A	2.22 ± 0.27 A
3% Beef Extract + 0.1M Sodium Citrate	2.88 ± 0.21 A	1.75 ± 0.33 A

Adsorption were conducted in the presence of the buffer that contained  $5.93 \pm 0.15 \log_{10}$  cfu/ml. *E. coli* and  $5.28 \pm 0.48 \log_{10}$  pfu/ml. MS-2. Buffers: 0.02 M imidazole and 0.02 M glycine, pH 7; all the chemical solutions were made in this buffer and were adjusted to pH 7. Values represent the mean and standard deviation for Triplicate determinations (5 g. 100 mesh sand treated with 1M FeCl<sub>3</sub> + 1M AlCl<sub>3</sub>).

For each column, values followed by the same letter were not significantly different then  $P > 0.05$  level.

Table 5-6. Effect of pH on removal of microorganisms by sand filters.

pH of Buffer	Uncoated Sand Log <sub>10</sub> Removal		Coated Sand Log <sub>10</sub> Removal	
	<i>E. coli</i>	MS-2	<i>E. coli</i>	MS-2
5	0.8 ± 0.1 A	1.6 ± 0.2 A	2.5 ± 0.4 C	4.8 ± 0.8 B
7	0.2 ± 0.2 C	0.1 ± 0.1 B	5.5 ± 0.1 A	6.1 ± 0.1 A
9	0.6 ± 0.4 B	0.2 ± 0.1 B	3.7 ± 0.4 B	5.2 ± 0.9 A, B

Values represent the mean and standard deviation of triplicate columns (10.0 x 2.5 cm, 80 g of 100 mesh sand). The titer of *E. coli* was 6.06, 5.52, and 5.94 log<sub>10</sub> CFR/ml in buffers of pH 5, 7, and 9, respectively. The titer of MS2 was 5.66, 6.19, and 6.49 log<sub>10</sub> PFU/ml in buffers of pH 5, 7, and 9, respectively.

#### Example 6 – Removal of Microorganisms by a Filter Containing Sand Coated With Metallic Hydroxides

**Modification of sand.** Silica Sand (Oglebay Norton, Brady TX) was purchased at a local pool supply store. For coating, the sand was soaked in a solution containing 0.2 M ferric chloride and 0.4 M aluminum chloride. After 30 minutes, excess solution was poured off and the sand was allowed to dry at 80° C. Occasional stirring can be used to break up the clumps that formed and to ensure that the sand was thoroughly dry. Next, the sand (while still warm) was added to 2 volumes of 3 M ammonium hydroxide and was allowed to soak for 10 minutes. The ammonium hydroxide was poured off, and the sand was dried again with occasional stirring at 80° C. The dried sand was then rinsed and stored at room temperature until used.

**Bacterial Cultures** The following bacterial strains were used in removal studies: *Escherichia coli* C-3000 (ATCC 15597), which was routinely grown in Tryptic Soy Broth (TSB) and assayed using MacConkey Agar (Difco Labs, Detroit, MI); and *Staphylococcus aureus* (ATCC 12600), which was grown on TSB and assayed on Manitol Salt Agar (Difco Labs, Detroit, MI).

**Viruses and Viral Assays.** Polio virus 1 (strain Lsc), Echo virus 1, and Coxsackie B5 virus were grown on buffalo green monkey (BGM) cells and assayed as plaque forming units (PFUs) by using an agar overlay technique (Smith and Gerba, 1982). Rotavirus SA11 was grown with serum free media on MA 104 Cells and assayed using Most Probable Number software (Environmental Protection Agency, 1994). The following bacteriophages and their host bacteria were used: MS2 (*Escherichia coli* C-3000, ATCC 15597); φX 174 (*Escherichia coli*, ATCC 13607); PRD-1 (*Salmonella typhimurium*, ATCC 19585). Bacteriophages were grown on their respective hosts and assayed by soft agar overlay (Snustad and Dean, 1971).

**Protozoan parasites** Viable oocysts of *Cryptosporidium parvum* were obtained from Waterborne Inc. (New Orleans, LA). For enumeration, Cryptoglow™ stain (Waterborne Inc, New Orleans, LA) was used according to the manufacturers instructions and the oocysts were visualized and counted under a fluorescence microscope.

**Filter set up and water samples.** Two identical filtration systems were used; one contained sand modified as described above and the other contained unmodified sand. Each filter system consisted of a

Porpoise 180 commercial swimming pool filter (Porpoise, Jacksonville, FL.) that was run by a 40 gal./min. timer controlled electrical pump connected to 100 gallon water container. Each filter contained 220 Kg of sand in a 0.05 M<sup>3</sup> area. Tap water was circulated through the filter for 5 hours per day during the 170 day test period. A total chlorine residual of 2 ppm was maintained in the water tanks while the filters were running. Ten times that residual was used for super chlorination at monthly intervals to mimic swimming pool treatment practices. Tank water was frequently changed, and samples were taken for metal analysis. Before seeding with bacteria or viruses, the tanks were drained and filled with water that had been dechlorinated by the addition of sodium thiosulphate. Chlorination was performed by the addition of household bleach. Residual chlorine was determined using O-tolidine (American Public Health Association, 1989).

*Microorganism removal* Throughout the test period bacteria, viruses and *Cryptosporidium* oocysts were added to 100 gallons of dechlorinated tap water to obtain a initial concentration of approximately 10<sup>5</sup>/ml. The tanks were mechanically agitated for 15 min and samples were taken before filtration. Fifty gallons of the seeded water was then passed through each filter and collected. Triplicate influent and effluent samples were assayed and used to determine the percent removals of the different microorganisms. In separate experiments, 100 gal of water were seeded with microorganisms and the water was circulated through the filter for one hour. Triplicate samples were taken out before and after circulation and percent removal of the test microorganisms was determined.

*Statistical analysis.* Standard deviations, correlations and general t test probabilities were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah).

*Water Metal analysis.* Metals in the water before and after filtering were analyzed by Inductive Coupled Argon Plasma Spectroscopy.

At the beginning of the study, both one pass experiments and 1 hour water recirculation tests were performed (Table 6-1). After one pass through the filter, the average removal of all viruses by the filter containing untreated sand was just above 20%. In contrast, after one pass through the filter with modified sand, the average viral removal of all viruses was over 60%. By allowing the water to circulate through the filter for 1 hour, the average removal of all viruses was over 99.5% by the filter with modified sand. The filter with untreated sand removed just over 30% of the test viruses. When the filters were challenged with bacteria, an average removal of 78% was obtained with the filter containing modified sand. In comparison, the filter with the unmodified sand removed 40% of the bacteria. *Cryptosporidium* oocysts were reduced by 51% in one pass experiments and by 95% following recirculation through the filter containing treated sand. Under the same conditions, percent reductions of 21% and 37% of *Cryptosporidium* oocysts were obtained by filters containing unmodified sand.

Table 6-1. Removal of microorganisms by Porpoise filters containing coated or uncoated sand. Both one pass experiments and water circulation through the filter experiments are shown*					
		One Pass Filtration		Water Circulation	
5	Microorganism	Coated	Uncoated	Coated	Uncoated
	SA 11	49%	23%	99.4%	38%
	Echo 1	63%	25%	99.8%	35%
	Polio 1	73%	27%	99.7%	39%
	Coxsackie B5	79%	30%	99.9%	45%
10	MS-2	53%	17%	99.98%	34%
	φX 174	69%	24%	99.9%	43%
	PRD-1	45%	8%	99.8%	49%
	<i>Cryptosporidium</i>	51%	21%	95%	36%
	<i>E. coli</i>	N/A	N/A	67%	35%
15	<i>S. aureus</i>	N/A	N/A	88%	44%

\*Data represent the average of triplicates. In all cases, the differences between removal by filters containing unmodified sand and filters containing modified sand were statistically significant ( $P < 0.05$ )

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The percent removal of various microorganisms did not drop significantly over the length of the test period. Each virus was assayed in triplicates. At day 170 the filter containing modified sand reduced the number of bacteriophage by 2.4  $\log_{10}$  units as compared to 3.0  $\log_{10}$  unit

25 Filter effluents always contained less than 0.01 mg/l of iron or aluminum ions even when the 50 gallons of water was circulated for 50 hours through the filter.

#### Example 7— Uncontrolled Precipitation of the Mixed Iron Oxides on Polyester Fabric Filters

30 Precipitation of metal hydroxides on both sand and fibrous media has been studied by many researchers. In this example, an *in situ* precipitation method can be used to coat iron oxides on the polyester fabric filters.

35 Several different procedures for treating the polyester fabric have been utilized. A preferred procedure involves soaking the polyester filters in  $\text{FeCl}_3$ , drying the filters overnight, and then precipitating the iron oxides, for example, with a strong ammonium hydroxide solution. The filters can then be dried in a vacuum oven. The preferred  $\text{FeCl}_3$  concentration was determined to be about 0.4 M. The preferred concentration of  $\text{NH}_4\text{OH}$  used was about 2 M. These concentrations were chosen to allow the maximum amount of coating to be deposited on the filter surface without clogging the filters. The selection of the above concentration was also based on visual observation of the uniformity of the treated

filters. The filters treated with the 0.4 M FeCl<sub>3</sub> appeared to be the most uniform. Filtration was performed using the apparatus and procedures described in Example 3, with the exception that each filter holder had four layers of the filters instead of one layer due to the high permeability of the fabric filters. The initial concentrations of *S. aureus* and *E. coli* were approximately  $2 \times 10^5$  and  $1 \times 10^5$  CFU/ml, respectively. Initial concentrations of the bacteriophages were  $1 \times 10^5$ ,  $1 \times 10^6$  and  $1 \times 10^4$  CFU/ml for MS2, PRD- 1 and  $\Phi$ X-174, respectively.

The zeta potential of the iron oxide coated filters is positive below pH 4. The isoelectric point of the treated filters is approximately 4.5. At the experimental pH, the treated filters are slightly less electronegative than the untreated filters. The treated filters were more wettable than the untreated filters.

10 The pressure drop of the untreated filters was 0.8 psi across 4 layers of filters and 3.3 psi across 24 layers of filters. The pressure drop of the iron oxide treated filters, on the other hand, was only 0.1 psi across 4 layers of filters and 1.2 psi across 24 layers of filters. The pressure drop of the treated filters was significantly lower than the untreated filters.

Results of the filtration of *S. aureus* and *E. coli* showed the filter coefficient of the iron oxide coated filters improves by only 18%, from 0.085 to 0.100, in the filtration of *S. aureus*. In the filtration of *E. coli*, the treated filters appear to show essentially no improvement at all. The treated filters have better performance in the filtration of bacteriophages. In the filtration of MS2, the filtration efficiency of the precipitated iron oxide coated filters is significantly higher than that of the untreated filters. The treatment or coating improved the filter coefficient by 500%. In the filtration of PRD- 1, the coating increased the filter coefficient by 516%. In the filtration of  $\Phi$ X- 174, the filter coefficient is even higher.

15 20 The filter coefficient of the treated filters in the filtration of  $\Phi$ X-174 is 0.08, which is an improvement over the untreated filters of 1,388%.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

25

Claims

- 1           1. A method of filtering a fluid, comprising the following steps:  
2           applying a surfactant or polymer to a filter's matrix; and  
3           bringing the filter's matrix in contact with a fluid, wherein removal of an impurity from the fluid  
4           is enhanced by the application of the surfactant to the filter's matrix.
- 1           2. The method according to claim 1, wherein said impurity is selected from the group consisting  
2           of: particles, dissolved chemical contaminants, ions, particulates, bacteria, viruses, protozoan parasites,  
3           fungus, yeast, submicron particles, chemical species, organic chemicals, inorganic chemicals, and dust.  
4
- 1           3. The method according to claim 1, wherein said surfactant or polymer is a cationic surfactant  
2           or polymer.
- 1           4. The method according to claim 1, wherein said surfactant or polymer is an anionic surfactant  
2           or polymer.
- 1           5. The method according to claim 1, wherein said surfactant or polymer is a nonionic surfactant  
2           or polymer.
- 1           6. The method according to claim 1, wherein a monomolecular of surfactant or polymer is  
2           applied to the filter's matrix.
- 1           7. The method according to claim 1, wherein the filter's matrix comprises at least one material  
2           selected from the group consisting of woven fabric, unwoven fabric, fiberglass, polypropylene, cellulose,  
3           sand, diatomaceous earth, fine sand, gravel, and particulate media.
- 1           8. The method of claim 1, wherein said fluid is selected from the group consisting of: water and  
2           air.
- 1           9. The method according to claim 3, wherein said cationic surfactant or polymer is selected from  
2           the group consisting of: C<sub>20</sub> TAB - C<sub>26</sub> TAB, wherein TAB is Trimethyl ammonium bromide, Poly (4-  
3           vinyl pyridin), Chitosan (Poly(d-glucosamine), Polyethylenimine, Hydrophobically modified cationic  
4           polymers, and Zwitterionic surfactants (+ group).

1           10. The method according to claim 4, wherein said anionic surfactant or polymer is selected from  
2 the group consisting of: Dicetyl phosphate, Phosphatidic acid, Poly (acrylic acid), Poly (butyl  
3 acrylate/acrylic acid), Hydrophobically modified anionic polymers and Zwitterionic surfactants.

1           11. The method according to claim 5, wherein said nonionic surfactant or polymer is polyvinyl  
2 alcohol.

1           12. The method according to claim 1, wherein a plurality of surfactants or polymers are applied  
2 to a corresponding plurality of filters' matrixes, and wherein each of the plurality of filters' matrixes are  
3 brought into contact with the fluid.

1           13. The method according to claim 12, wherein at least one of the plurality of surfactants or  
2 polymers is cationic and at least one other of the plurality of surfactants or polymers is anionic.

1           14. A method of filtering a fluid, comprising the following steps:  
2           *in situ* precipitating at least one metallic (hydr)oxide to the filter's matrix; and  
3           bringing the filter's matrix in contact with a fluid, wherein removal of at least one impurity from  
4 the fluid is enhanced by the application of the at least one metallic (hydr)oxide to the filter's matrix.

1           15. The method according to claim 15, wherein the filter's matrix is heated to an elevated  
2 temperature during the step of *in situ* precipitating at least one metallic (hydr)oxide to the filter's matrix.

1           16. The method according to claim 15, wherein the elevated temperature is between about 60°C  
2 to about 100 °C.

1           17. The method according to claim 15, wherein the elevated temperature is between about 70°C  
2 to about 95 °C.

1           18. The method according to claim 15, wherein the elevated temperature is between about 75°C  
2 to about 85°C

1           19. The method of claim 15, wherein the elevated temperature is about 80 °C.

1           20. The method according to claim 14, wherein the step of *in situ* precipitating at least one  
2 metallic (hydr)oxide to a filter's matrix comprises the steps of:  
3           applying at least one of the group consisting of: metallic chloride and metallic sulfate;  
4           drying the filter's matrix;  
5           applying a base solution to the filter's matrix; and

6 drying the filter's matrix.

1 21. The method according to claim 20, wherein said at least one metallic chloride is selected  
2 from the group consisting of ferric chloride and aluminum chloride is applied to the filter's matrix.

1 22. The method according to claim 21, wherein the filter's matrix is heated to an elevated  
2 temperature during the application of the at least one metallic chloride.

1 23. The method according to claim 22, wherein the filter's matrix is agitated during the  
2 application of the at least one metallic chloride.

1 24. The method of claim 22, wherein said at least one metallic sulfate is selected from the group  
2 consisting of: ferric sulfate and aluminum sulfate is applied to the filter's matrix.

1 25. The method according to claim 24, wherein the filter's matrix is heated to an elevated  
2 temperature during the application of at least one metallic sulfate.

1 26. The method according to claim 25, wherein the filter's matrix is agitated during the  
2 application of the at least one metallic sulfate.

1 27. The method according to claim 21, wherein said at least one metallic chloride is misted onto  
2 the filter's matrix.

1 28. The method according to claim 24, wherein said at least one metallic sulfate is misted onto  
2 the filter's matrix.

1 29. The method according to claim 20, wherein said base is selected from the group consisting  
2 of: ammonium hydroxide, sodium hydroxide, and potassium hydroxide.

1 30. The method according to claim 20, wherein at least one of the drying steps is performed at  
2 an elevated temperature.

1 31. The method according to claim 20 wherein said at least one metallic chloride comprises ferric  
2 chloride and aluminum chloride.

1 32. The method according to claim 31, wherein the ferric chloride is in the range of 0.1 M to 2.0  
2 MN.

- 1           33. The method of claim 31, wherein the ferric chloride is in the range of 0.2 M to 0.4 M.
- 1           34. The method according to claim 31, wherein the ferric chloride is about 0.25 M.
- 1           35. The method according to claim 31, wherein the aluminum chloride is in the range of 0.1 to  
2   2.0 M.
- 1           36. The method according to claim 31, wherein the aluminum chloride is in the range of .02 M  
2   to 0.8 M.
- 1           37. The method according to claim 31, wherein the aluminum chloride is about 0.5 M.
- 1           38. The method according to claim 20, wherein the metallic chloride solution comprises water  
2   and ethanol.

# INTERNATIONAL SEARCH REPORT

International Application No.  
**PCT/US 99/20352**

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 B01D37/02**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
**IPC 7 B01D**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 550 950 A (SANWA KAGAKU KENKYUSHO CO.) 14 July 1993 (1993-07-14) page 2, line 45 -page 3, line 30	1,2,5,7, 12
X	EP 0 056 117 A (INSTITUT DE RECHERCHES HYDROLOGIQUES) 21 July 1982 (1982-07-21) claims 1-7	14
A	US 4 652 379 A (F.NYBERG) 24 March 1987 (1987-03-24) column 9, line 31 -column 10, line 8; claim 1	1,14,25
A	EP 0 287 917 A (THE GRAVER COMP.) 26 October 1988 (1988-10-26) claim 1; example 1	1-8,13
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

**11 February 2000**

Date of mailing of the international search report

**24/02/2000**

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentkan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

**Bertram, H**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 99/20352

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 4, no. 93, 5 July 1980 (1980-07-05) & JP 55 056814 A (MITSUBISHI HEAVY IND.), 26 April 1980 (1980-04-26) abstract	1,11
A	PATENT ABSTRACTS OF JAPAN vol. 1996, no. 2, 29 February 1996 (1996-02-29) & JP 07 256025 A (KENJI NAKAMURA), 9 October 1995 (1995-10-09) abstract	1,9
A	DATABASE WPI Week 199704 Derwent Publications Ltd., London, GB; AN 1997-041127 XP002130239 & RU 2 058 812 C (UYOD), 27 April 1996 (1996-04-27) abstract	20,29
A	PATENT ABSTRACTS OF JAPAN vol. 17, no. 489, 6 September 1993 (1993-09-06) & JP 05 123513 A (MITSUBISHI PAPER MILLS), 21 May 1993 (1993-05-21) abstract	1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/20352

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 550950 A	14-07-1993	JP 5188053 A US 5364533 A	27-07-1993 15-11-1994
EP 56117 A	21-07-1982	FR 2497685 A	16-07-1982
US 4652379 A	24-03-1987	BR 7908985 A DK 188080 A EP 0016220 A FI 792764 A JP 55501013 T NO 792880 A SE 7809355 A WO 8000542 A SE 8200903 A	30-12-1980 30-04-1980 01-10-1980 06-03-1980 27-11-1980 06-03-1980 06-03-1980 03-04-1980 17-08-1983
EP 287917 A	26-10-1988	US 4747955 A CA 1319327 A MX 168307 B	31-05-1988 22-06-1993 17-05-1993
JP 55056814 A	26-04-1980	JP 1433617 C JP 62040053 B	07-04-1988 26-08-1987
JP 07256025 A	09-10-1995	NONE	
RU 2058812 C	27-04-1996	NONE	
JP 05123513 A	21-05-1993	NONE	